In Vitro Cytotoxicity Evaluation of a Self-adhesive, Methacrylate Resin–based Root Canal Sealer

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Abstract

This study compared the cytotoxicity of MetaSEAL (Parkell Inc, Farmington, NY), a methacrylate resin-based sealer with an epoxy resin-based (AH Plus Jet; Dentsply Caulk, Milford, DE) and a zinc oxide–eugenol-based sealer (Pulp Canal Sealer; SybronEndo, Orange, CA). Five-millimeter diameter disks prepared from the respective sealer and disks prepared from Teflon (negative control) and polymethyl methacrylate (positive control) were placed in direct contact with a rat osteosarcoma (ROS) 17/2.8 rat osteoblast-like cell line at six intervals after setting completely at 72 hours and for 5 succeeding weeks after the disks were immersed in simulated body fluid. Succinate dehydrogenase activity was evaluated by using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay. All sealers exhibited severe toxicity at 72 hours, after which toxicity decreased gradually over the experimental period except for Pulp Canal Sealer, which remained severely toxic. MetaSEAL was more toxic than AH Plus Jet during the first week. Both were similar to the toxicity profile of the positive control after the first week, which was probably diffusion controlled. *(J Endod 2008; 34:1085–1088)*

Key Words

Cell culture, cytoxicity, MetaSEAL, succinate dehydrogenase, 3-(4;5-dimethylthiazole-2-yl)-2;5-diphenyl tetrazolium bromide assay

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The use of a root canal sealer with a thermoplastic core filling material is considered the standard of care in the obturation of root canal spaces (1). Although sealers are intended to be contained within the root canal (2–4), they sometimes inadvertently extrude through the apical constriction during placement (5–7). Even without extrusion, eluents derived from these materials (8–10) may come into contact with periradicular tissues (11), with potential irritation of the periradicular tissues that results in delayed wound healing (12, 13). Thus, the biocompatibility of root canal sealers and filling materials are critical to the clinical success of endodontic therapy (14–16).

Currently used sealers are based on calcium hydroxide, zinc oxide–eugenol, diketone, polydimethylsiloxane, glass ionomer, epoxy resin, or methacrylate resin. They exhibit a variable degree of cytotoxicity depending on the conditions under which testing was performed (17). Most in vitro cytotoxicity tests were designed to screen the biologic responses of root canal sealers for short periods (freshly mixed, 14 days) (18). These intervals are probably inadequate to predict the biologic responses of extruded sealers that remain in contact with periapical tissues for decades. Very few studies had attempted to examine the longitudinal cytotoxic behavior of root canal sealers (19, 20).

A self-adhesive, dual-curable methacrylate resin-based sealer (MetaSEAL; Parkell Inc, Farmington, NY) has recently been introduced commercially. This sealer is also marketed as Hybrid Bond SEAL in Japan (Sun Medical, Shiga, Japan) (21). The sealer purportedly bonds to thermoplastic root-filling materials as well as radicular dentin via the creation of hybrid layers in both substrates. The sealer is self-etching and hydrophilic because of the inclusion of an acidic resin monomer 4-methacryloyloxyethyl trimellitate anhydride and is recommended for use exclusively with cold compaction or single-cone techniques (22). It has been described by the manufacturer as highly biocompatible and well tolerated by connective tissues.

The objective of the present study was to assess the in vitro cytotoxicity of MetaSEAL over longer time periods and compare the results with an epoxy resin-based sealer and a zinc oxide–eugenol-based sealer. The rationale for the study was that these longer-term tests provide a more extensive toxicity profile that would be useful in predicting the clinical performance of the methacrylate resin–based sealer. The null hypothesis tested was that there is no difference in the in vitro cytotoxicity exhibited by the three sealers over a 5-week period of immersion in a simulated body fluid (SBF).

Materials and Methods

Specimen Preparation

Three endodontic sealers were evaluated: MetaSEAL, AH Plus Jet (Dentsply Caulk, Milford, DE), and Pulp Canal Sealer (SybronEndo, Orange, CA). The compositions of these sealers are shown in Table 1. The sealers were mixed according to the manufacturers' instructions under aseptic conditions to limit the risks of microbial contamination (23). Components A and B of AH Plus Jet were mixed by using the automixing syringe. Pulp Canal Sealer was prepared by hand mixing powder and liquid until a homogeneous consistency was obtained. MetaSEAL was hand mixed using one scoop of powder and three drops of liquid for 30 seconds.

Each material was packed into six presterilized Teflon molds (3 mm thick × 5 mm diameter) and covered on both sides with presterilized Mylar sheets ($N = 6$). A previous study established that six replicates would provide sufficient statistical power to

TABLE 1. Composition of the Three Root Canal Sealers Examined in the Present Study

Root Canal Sealer	Components	
MetaSEAL	Powder: zirconium oxide fillers, amorphous silica fillers, polymerization initiator	Liquid: 60% 4-META, 40% HEMA and dimethacrylates
AH Plus Jet	Component A: epoxy resin, calcium tungstate, zirconium oxide, aerosil, iron oxide	Component B: adamantane amine, N,N-dibenzyl-5-oxanonane, TCD-diamine, calcium tungstate, zirconium oxide, aerosil
Pulp Canal Sealer	Powder: 34% zinc oxide, 25% silver, 30% resins, 11% thymol iodide	Liquid: Canada balsam, eugenol

4-META = 4-methacryloyloxyethyl trimellitate anhydride; HEMA = 2-hydroxyethyl methacrylate.

detect changes in toxicity of <10% given the magnitude of standard deviations when $\alpha = 0.05$ (23). The molds were also covered with a glass slab on each side and are clamped to spread the sealers and exclude oxygen from the methacrylate resin–based sealer. MetaSEAL was polymerized through the glass slab for 2 minutes from each side using a light-curing unit with an output intensity of 600 mW/cm^2 (Optilux 500; Demetron Research Corporation, Danbury, CT). The specimens were allowed to set for 72 hours at 37°C and 100% humidity under sterile conditions. Teflon and autopolymerized poly(methyl methacrylate) (24) disks with the same dimensions were used as the respective negative and positive controls.

Cell Culture

Materials were tested for cytotoxicity by using a ROS 17/2.8 rat osteoblast-like cell line (25–27) because they have been well characterized, are reproducible, and serve as a good initial screening model for evaluation of the biocompatibility of dental cements and how these cements affect bone formation. ROS 17/2.8 osteoblast-like cells originate from rat bone sarcoma and have been reported to contain a large proportion of proliferative, phenotypically immature cells that closely resemble progenitor osteoblast cells (28). These osteosblast-like cells were raised in tissue-culture flasks and incubated a culture medium for 7 days at 37°C in a humidified 95% air–5% CO_2 atmosphere until the cells become fully established before they are used for testing. An F-12 medium (Gibco, Invitrogen Corp, Carlsbad, CA) was used and supplemented with 28 mmol/L HEPES (Calbiochem, La Jolla, CA), 1.1 mmol/L $CaCl_2$ (Allied Chemical, Moristown, NJ), 5% NuSerum (Collaborative Res, Bedford, MA), and 25 mmol/L L-glutamine and 125 U/mL penicillin-streptomycin (Gibco). The cells were plated at 40,000 cells/cm^2 in 0.5 mL in a 24-well format.

Cytotoxicity of the three endodontic sealers was assessed after the initial 72-hour setting period (ie, week 0) and for five succeeding weeks, following the method reported by Brackett et al. (29). One specimen was placed in the center of each well and secured so that the specimen could not move (23). The surface area-to-volume ratio of the specimen to medium was approximately 150 mm^2/mL (within International Organization for Standardization (ISO) 10993 specifications) (30). Between tests, the specimens were aseptically removed and rinsed twice with sterile SBF. The SBF was prepared by dissolving 136.8 mmol/L NaCl, 3.0 mmol/L KCl, 2.5 mmol/L $CaCl_2 \cdot 6H_2O$, 1.5 mmol/L $MgCl_2 \cdot 6H_2O$, 0.5 mmol/L $Na_2SO_4 \cdot 10H_2O$, 4.2 mmol/L $NaHCO_3$ and 1.0 mmol/L $K_2HPO_4 \cdot 3H_2O$ in deionized water, buffered to pH 7.4 with 0.1 mol/L Tris Base and 0.1 mol/L HCl, and autoclaved. Each specimen was then immersed for 4 days in 10 mL SBF before securing in a new cell-plated well and incubated for another 3 days at 37°C and 5% CO_2 atmosphere before the next assay cycle.

Cytotoxicity Testing

Cell mitochondrial activity was determined by estimating their succinate dehydrogenase (SDH) activity using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (31). Briefly, the sealer specimens and the culture medium were removed from each well. The remaining cells were gently washed with 1.0 mL of phosphate-buffered saline (pH 7.4). The wash was replaced with an MTT-succinate solution (1 mg/mL MTT and 2.0 molal disodium succinate) for 60 minutes at 37°C. The reaction was then quenched, and the cells were fixed by adding 0.5 mL Tris-formalin solution (0.2 mol/L Tris, 4% formalin, pH 7.2) for 2 to 3 minutes, after which all solution was removed and the cell monolayer allowed to dry for 5 to 10 minutes.

After drying, the cell monolayer was washed with 1 mL deionized water. The MTT-formazan in the cells was dissolved in situ using a dimethyl sulfoxide (DMSO)-NaOH solution (6.25% v/v 0.1 N NaOH in DMSO). A 100-μL aliquot of the solution was transferred to a 96-well tray, and the optical density was measured by using a VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA) at 562 nm. The optical densities of the blank solutions (DMSO-NaOH only) were subtracted from all wells. The formazan content of each well was computed as a percentage of the Teflon controls on each culture plate. Cytotoxicity responses were qualitatively rated as severe (<30%), moderate (30%–60%), slight (60%–90%), or noncytotoxic (>90%) relative to the SDH activity of the Teflon controls (29) as well as analyzed quantitatively.

Statistical Analysis

Because the normality and homoscedasticity assumptions of the data were violated, they were analyzed by using nonparametric statistical methods. For each material, differences in SDH activity over the six time intervals were analyzed by using repeated analysis of variance on ranks. For each time period, differences in SDH activity were analyzed by using Kruskal-Wallis analysis of variance. Post hoc multiple comparisons were performed by using Dunn's tests. All statistical significances were preset at $p = 0.05$.

Results

Results of the MTT assay over the six time periods are listed in Table 2 and graphically represented in Figure 1. The three sealers appeared severely toxic when they were evaluated at 72 hours after mixing (week 0) and were not significantly different from the positive control ($p > 0.05$). MetaSEAL remained severely cytotoxic at week 1, was mildly cytotoxic at weeks 2 and 3, and became noncytotoxic after week 3. AH Plus was similar to the positive control in that it was rendered moderately cytotoxic after the first cycle of SBF immersion (week 1), became only mildly cytotoxic at weeks 2 and 3, and was noncytotoxic after week 3. MetaSEAL was significantly more cytotoxic than AH Plus Jet and the positive control during week 1 ($p < 0.05$), but its toxicity was not significantly different from these two materials at weeks 2 to 5 ($p > 0.05$). Conversely, Pulp Canal Sealer remained severely cytotoxic and with no significant decrease in its toxicity ($p > 0.05$) over the entire experimental period. At the end of the fifth week, Pulp Canal Sealer